Mammary Gland Neoplasia

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Determining how findings of chemically induced carcinogenic effects in rodents can properly be interpreted for human health poses a continuing challenge to the risk assessment community. One approach begins by comparing and contrasting carcinogenic processes in rodents and humans, identifying biologically significant similarities and differences and gaps in scientific knowledge and understanding. Russo and Russo (in this issue) use just such an approach to evaluate the current state of scientific understanding of the comparative mechanisms of mammary tumorigenesis in humans and rodents, particularly the role of reproductive hormones. This commentary describes the basis for this review and suggests some of the implications the report may have for human health risk assessment and for future research. *Key words*: breast cancer, chemical carcinogens, estrogen, hormones, interspecies extrapolation, mammary tumors, risk assessment, rodent bioassay. *Environ Health Perspect* 104:912–914 (1996)

During 1992, the International Life Sciences Institute's Risk Science Institute, in partnership with the U.S. Environmental Protection Agency (EPA), Office of Pesticide Programs (OPP), formed a working group to examine the relationship(s) between chemically induced mammary tumors in laboratory rodents and human breast cancer. Specifically, the working group composed of scientists from government, industry, and academia was asked to consider five questions: Is a dose-related increase in mammary tumor incidence in rodents a credible indicator for potential human risk? Is the mechanism of tumor development partially or completely mediated by hormones? Does the mechanism of carcinogenic response function with or without a threshold? How do various routes of exposure (e.g., oral, inhalation, subcutaneous) affect rodent mammary carcinogenesis and how should such information be used to select an appropriate animal model for human risk assessment? What information and methodology should be used to estimate potential human risk?

Through subsequent meetings and discussions, it became clear that a fundamental barrier to addressing these questions was the absence of data on the initiation and progression of chemically induced mammary tumors in women. It also became apparent that a thoughtful comparative analysis of the role that reproductive hormones play in rodent mammary tumorigenesis and human breast carcinogenesis would be critical to addressing such questions. If modes of action, modulatory activities, and temporal influences of hormones on tumorigenic processes could be established, hormonal effects could potentially be separated from chemical effects, which would facilitate broader understanding of mammary tumorigenesis. Indeed, the late Eugene Paynter of the OPP initiated just such a review during the late 1980s. Using Paynter's work as a starting point, Irma Russo accepted the working group's invitation to undertake this critical review of the state of the science and to discuss those factors that might be relevant to resolving these issues.

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The accompanying report, "Mammary Gland Neoplasia in Long-term Rodent Studies," by Russo and Russo (*1*) is the result of this effort. It carefully examines the developmental biology of the mammary gland in rodents and in humans in the absence of known exposure to xenobiotic chemicals, noting that there are distinct anatomical and developmental differences between the mammary glands of rats, mice, and humans. These differences become more apparent when the influences of parity, lactation, gland involution, and other physiologic processes on the tissue are considered.

When the influence of reproductive and other hormones is examined, the differences between the mammary glands of rodents and humans are even more evident. Rodent studies employing ovariectomy and hormonal replacement therapy underscore the importance of the endocrine milieu in mammary gland development and involution.

Hormone-dependent neoplasia varies significantly between species and among strains within a species. The incidence of mammary tumors among untreated female rats is variable, 20%–60% after 2 years (*2*). In contrast, the overall lifetime incidence of breast cancer among women in the United States is about 12% (*3*). Female rats and humans develop mammary tumors of unknown etiology that are hormonally regulated to various degrees. Although these types of mammary tumors in mice also are susceptible to endocrine-mediated regulation, most are associated with infection by mouse mammary tumor virus. Rodent mammary gland tumors seldom metastasize, reflecting another fundamental difference between most human breast cancer and rodent mammary tumors.

From an experimental perspective, the long latency and variable incidence of mammary tumors among untreated female rats limits the practical utility of studying such tumors as models of human disease. Genotoxic agents such as dimethylbenz(*a*)-anthracene (DMBA), *N*–methyl–*N*–nitrosourea (MNU), and 3–methylcholanthrene (MCA) are among the most commonly used compounds to elicit mammary gland tumors in rodents. These agents are thought to act as initiators within the context of the multistage model of carcinogenesis. Tumor promotion in mammary tissue may be hormonally mediated because the expression of tumorigenicity following initiation with such genotoxic agents is determined, at least in part, by age-related endocrine kinetics.

In mice, tumors induced by such compounds have a long latency and require multiple exposures. In contrast, such tumors can be elicited in rats after a single exposure during a critical period in postnatal development and have a much reduced latency period. The incidence and number of chemically induced tumors vary with dose and route of exposure and between different strains of rats administered identical doses by identical routes. Although there are certain anatomic, histologic, and developmental similarities between the mammary glands of rats and humans, there is little

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or no direct evidence that these or other genotoxic agents do or do not induce breast cancer in women. However, such compounds can induce mutations and/or neoplastic transformation of human breast epithelial cells *in vitro*.

Epidemiological studies have generally failed to identify specific chemical exposures or other factors that contribute substantively to the likelihood of developing breast cancer. Because breast cancer risk is associated with nulliparity, late first full-term pregnancy, early menarche, late menopause, and exposure to ionizing radiation at a young age (*4*), some have suggested that breast cancer may be initiated by exposure to carcinogenic compounds during a narrow window of opportunity extending from menarche until the time of the first full-term pregnancy (*5*). Studies of chemical exposures of susceptible subpopulations of women, e.g., those expressing the BRCA1 (*6*) or BRCA2 (*7*) gene products, may provide the opportunity to better understand the relationship between human breast cancer and chemical exposure.

Underlying all such generalizations is an extensive and complex literature characterizing the modulatory effects of reproductive and other hormones on all aspects of mammary gland growth, development, and disease. A plethora of endocrine interactions can permanently affect mammary gland structure, organization, and function as well as altering, at least in rodents, the response to exposure to mammary tumor-inducing chemicals. Ovarian and placental hormones, pituitary and thyroid hormones, androgens, and insulin have all been demonstrated to affect the tumorigenic responses of rats exposed to genotoxic mammary carcinogens. Similarly, various growth factors, e.g., inhibin, fibroblast growth factors, and other cytokines, can modulate the development and growth of chemically induced mammary tumors in rats. Although the complex hormonal environments associated with the rat estrous cycle and the human menstrual cycle differ in detail, the modulatory effects of rat hormones on mammary tumor development and growth raise the possibility of similar hormonal modulation of human breast cancer. Indeed, studies of women undergoing ovariectomy, estrogen replacement therapy, and tamoxifen therapy as a treatment for breast cancer provide evidence of endocrine involvement in human disease.

Relevance for Risk Assessment

The impetus for the review by Russo and Russo (*1*) was to evaluate the relevance of findings of chemically induced mammary tumors in rodents for human health risk assessment. To address this issue, it is appropriate to consider their findings in light of the questions and issues raised with the working group.

Is a dose-related increase in mammary tumor incidence in rodents a credible indicator for potential human risk? In the absence of evidence to the contrary, the EPA considers findings of mammary tumors in chemical-exposed rodents indicative of the potential of the chemical to cause cancer in humans (e.g., breast cancer in women). Although the report by Russo and Russo (*1*) identifies certain differences as well as similarities between the mammary glands of rodents and humans, these findings do not substantively challenge the EPA's current assumption. The paucity of human data with respect to chemical exposure precludes determination of whether such findings are truly predictive of human risk. The issue is further confounded by the observation that most of the chemicals that elicit mammary gland tumorigenesis in rodents are genotoxic agents and represent only a small fraction of the compounds to which women are likely to be exposed. From a risk assessment perspective, consideration of factors including dose response, route of administration, and mode/mechanism of action will influence assessment of the relevance of the results of the animal studies for predicting the response of women. Relevance also will be influenced by the type, incidence, number, and size of the chemically induced tumors and the occurrence of tumors in other tissues and organs.

Is the mechanism of mammary tumor development partially or completely mediated by hormones? Russo and Russo (*1*) indicate that mammary tumorigenesis can be modulated by hormones. Although there is evidence that ovariectomy and the subsequently ablated hormonal microenvironment can inhibit tumor development and growth, the synergistic, agonistic, and antagonistic relationships among the various hormones that are known to influence rodent mammary tumors suggest that they are modulatory in effect. There is clearly a need to better understand the basis for such modulatory effects and the implications that hormonally modulated rodent mammary tumors have for breast cancer in women.

Does carcinogenic response operate with or without a threshold? The report by Russo and Russo (*1*) does not address this issue. This concern reflects the observation that estrogen and other hormones which modulate mammary tumorigenesis operate through receptors. In at least some ligand/receptor systems, a threshold number of receptors must be occupied to elicit a full biologic response. Whether or how this applies in the consideration of chemically induced mammary tumors remains to be determined. Although mammary carcinogens may act through the estrogen or other hormone receptors, endocrine disruption may occur during hormone synthesis, secretion, or transport, or at the level of second messenger activation or function (*8*). The uncertainty in our understanding of the relationships between the estrogen receptor and its various agonistic and antagonistic ligands is illustrative of the complexity of this issue. For example, the responses of cells bearing mutated estrogen receptors may be stimulated by tamoxifen but inhibited by estradiol (*9*).

How do various routes of exposure affect rodent mammary tumorigenesis and how should such information be used to select an appropriate animal model for human risk assessment? Various routes of administration have been used in the study of rodent mammary tumorigenesis. In the case of DMBA, tumors are typically induced by intragastric administration, while MNU-induced tumors can be induced by intravenous or subcutaneous administration. However, carefully selected doses of DMBA and MNU administered by different routes to animals of a single strain elicit similar incidences of tumors with similar latency periods. Such observations suggest that various routes of exposure may be acceptable for hazard characterization studies, but the evidence is limited with respect to the number and types of compounds considered. Ideally, the route(s) of exposure used during rodent studies to assess the carcinogenic potential of chemicals should reflect the most likely or significant route of potential human exposure. Given the dearth of information about chemically induced human breast cancer, it seems premature to select a single animal model or route for use in hazard identification studies. Even suggesting combinations of models and routes would be more speculative than informed.

What information and methodology should be used to estimate potential human risk? Clearly there is a need to make better use of human data by designing better studies or asking better questions of existing databases (*10*). Occupational exposure studies may help us to identify and understand some aspects of chemically induced breast cancer. Perhaps more information can be extracted from rodent carcinogenicity studies that would facilitate interpretation of the results. Better characterization of test compound pharmacokinetics and pharmacodynamics would enhance under-

standing. Outside of the current 2-year carcinogenicity bioassay, further animal studies of the etiologic factors associated with carcinogenicity in various test species may lead to improved assessment of breast cancer risk in women (*11*). For example, normal human breast tissue might be transplanted into immunologically incompetent rodents to determine whether exposure to certain chemicals elicits the tumorigenic response observed in normal mice exposed to the same chemicals. Performing biologically based dose–response studies in rodents following full-term pregnancy, lactation, and mammary gland involution might illuminate the apparent protective effects of pregnancy and lactation in humans. Testing chemicals in multiple strains of rodents, including those with low and high incidences of spontaneous mammary gland neoplasms, may elicit greater appreciation for, and understanding of, variability in the tumorigenic response. Pharmacokinetic studies performed using human breast tissue *in vitro* and with rodents both *in vivo* and *in vitro* may allow the use of the parallelogram approach to risk assessment (*12*). *In vitro* studies of the molecular biology and biochemistry of hormone receptors with respect to measurable physiologic endpoints, e.g., cell proliferation or cell activation, are valuable in understanding and validating the use of *in vivo* and *in vitro* estrogen screening assays (*13*). Such studies could provide more satisfying answers to the simply stated but complex questions raised earlier.

Russo and Russo (*1*) bring together a wide array of observations and information about mammary gland tumors in rodents and humans. The resulting synthesis may help to channel research efforts and resources along lines that will ultimately provide insight into the difficult issues that confront the risk assessment community relative to the interpretation of the findings of mammary tumors in rodents.

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